

CLAIMS

1. A method of reconstituting an animal embryo, the process comprising
5 transferring a nucleus into a first oocyte followed by removing and transferring said
nucleus from said oocyte to a further oocyte or to an enucleated fertilised zygote.
2. A method as claimed in claim 1, wherein the first oocyte is a mature
10 metaphase II oocyte (unfertilised egg) or an activated MII oocyte.
3. A method as claimed in claim 1 or claim 2, wherein the further oocyte is an
enucleated MII oocyte.
4. A method as claimed in any one of claims 1 to 3, in which the reconstructed
15 embryo is cultured *in vitro* or *in vivo* to a stage suitable for transfer to a final
surrogate recipient for development to term.
5. A method as claimed in any one of claims 1 to 3, in which the reconstructed
embryo is transferred to a final surrogate recipient to support embryo development
20 and development to term.
6. A method as claimed in any one of claims 1 to 5, in which the donor nucleus is
genetically modified.
7. A method as claimed in any of claims 1 to 7, wherein a diploid cell donates the
25 nucleus.
8. A method as claimed in any one of claims 1 to 7, wherein the diploid nucleus
is donated by a G1 cell.
9. A method as claimed in any one of claims 1 to 7, wherein the diploid nucleus
30 is donated by a cell arrested at the G1/S-phase border.

10. A method as claimed in any one of claims 1 to 6, wherein the nucleus is donated by a tetraploid cell
- 5 11. A method as claimed in any one of claims 1 to 6, wherein the tetraploid nucleus is donated by a G2 cell
12. A method as claimed in any one of claims 1 to 11, wherein the tetraploid nucleus is donated by a mitotic cell.
- 10 13. A method as claimed in any one of claims 1 to 6, wherein the nucleus is donated by a cell of unknown ploidy.
14. A method as claimed in any one of claims 1 to 6 or claim 13, wherein the nucleus of unknown ploidy is donated by a growing cell at any point in the cell cycle i.e. G1, S, G2 or M.
- 15 15. A method as claimed in any one of claims 1 to 6, wherein the nucleus is donated by a cell arrested at any point in the cell cycle i.e. G0, G1, G1/S, S, G2 or M by any means
- 20 16. A method as claimed in any one of claims 1 to 15, wherein the recipient oocyte for the first nuclear transfer is enucleate.
- 25 17. A method as claimed in any one of claims 1 to 16, wherein the first nuclear transfer is achieved by cell fusion, or by cell or nuclear injection.
18. A method as claimed in any one of claims 1 to 17, in which the animal is an ungulate species.
- 30 19. A method as claimed in any one of claims 1 to 18, wherein the animal is a cow or bull, pig, sheep, goat, camel, waterbuffalo.

20. A method as claimed in any one of claims 1 to 17, wherein the animal is a mouse, rat or other rodent.

21. A method as claimed in any one of claims 1 to 17, wherein the animal is a lagomorph.

22. A method as claimed in claim 21, wherein the animal is a rabbit.

23. A method as claimed in any one of claims 1 to 22, wherein correct ploidy is maintained by combination of donor and recipient cell cycle stage.

24. A method as claimed in any one of claims 1 to 23, wherein correct ploidy is maintained by treatment of the reconstructed embryo with any compound/s that maintain correct ploidy.

25. A method as claimed in any of the claims 1 to 24, wherein the donor nucleus is then transferred to a second recipient cell.

26. A method as claimed in any claim 1-25 wherein the second recipient cell is a fertilised zygote.

27. A method as claimed in any claim 1-25 wherein the second recipient cell is an activated oocyte.

28. A method as claimed in any of the claims 1-27 wherein the second recipient cell is enucleated.

29. A method of preparing an animal, the method comprising:

(a) reconstituting an animal embryo as claimed in any one of the preceding claims;

- (b) causing a foetus to develop from the embryo; and
- (c) causing an animal to develop to term from the foetus; and
- (d) optionally, breeding from the animal so formed.

5 30. A method as claimed in claim 29, wherein the animal embryo is further manipulated prior to full development of the embryo.

31. A method as claimed in claim 29, wherein the animal foetus is further manipulated prior to full development of the embryo.

10 32. A method as claimed in any one of claims 29 to 31, wherein a new cell line is derived from the reconstructed embryo.

15 33. A method as claimed in any one of claims 29 to 31, wherein a new cell line is derived from the animal foetus prior to full development of the embryo.

34. A method as claimed in any one of claims 29 to 31, wherein a new cell line is derived from the resultant animal.

20 35. A method as claimed in any one of claims 29 to 34, wherein more than one animal is derived from the embryo.

36. A reconstituted animal embryo, which is capable of giving rise to a live birth and is prepared by a method as claimed in any one of claims 1 to 35.

25 37. An animal obtainable or obtained by a method as claimed in any one of claims 1 to 36.

30 38. An animal obtainable or obtained from an embryo as claimed in any one of claims 1 to 36.

39. An embryonic stem cell line obtainable or obtained from an embryo produced by any one of claims 1-28.

5 40. An undifferentiated cell line or population obtainable or obtained from an embryo produced by any one of the claims 1-28.

41. A differentiated cell line or population obtainable or obtained from an embryo produced by any one of the claims 1-28.

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